



Bioremediation Approaches and Tools for Anaerobic Benzene Remediation



Sandra Dworatzek
SiREM

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Toronto, ON
January 26, 2017

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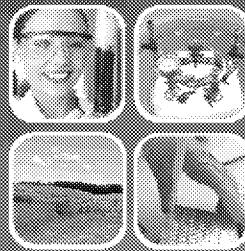
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Bioremediation Approaches and Tools for Anaerobic Benzene Remediation

Phil Dennis, Jeff Roberts, Sandra Dworatzek and Peter Dollar,
SIREM Guelph, Ontario
Elizabeth Edwards and Fei Liu, University of Toronto, Toronto,
Ontario

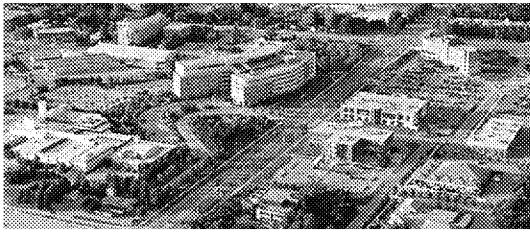


Presented by:
Sandra Dworatzek,
SIREM

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■ ■ ■ Introduction to SIREM

- Founded in 2001 to provide laboratory testing services and products for site remediation
- Located in the University of Guelph Research Park
- More information www.siremlab.com



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University of Guelph Research Park

Remediation Approaches

Category	Technology	Example Target Contaminants
Aerobic	Oxygen Addition	Petroleum Hydrocarbons, Pesticides
	Nutrient Addition	
	Bioaugmentation	Petroleum Hydrocarbons, Pesticides
Anaerobic	Electron Donor Addition	Chlorinated Solvents, Perchlorate, Oxidized Metals, Explosives, Nitrate
	Bioaugmentation (KB-1® / KB-1® Plus/ DGG-1)	PCE, TCE, DCE, VC and 1,2-DCA Chlorinated ethanes and methanes such as 1,1,1-TCA, carbon tetrachloride and chloroform; CFC-113 Benzene
	Electron Acceptor Addition	Petroleum Hydrocarbons
Cometabolic	Gas infusion, Bioaugmentation	1,4-Dioxane, NDMA, Chloroform, TCE, DCE, VC, MTBE, Creosote, >300 different compounds
Abiotic	Natural Attenuation Reduced Metals	Chlorinated solvents, Oxidized metals,



Anaerobic vs. Aerobic Respiration

Aerobic respiration

metabolic reactions and processes that take place in the cells of organisms that use oxygen as the terminal electron acceptor

Anaerobic respiration

metabolic reactions and processes that take place in the cells of organisms that use electron acceptors other than oxygen (e.g., sulfate)





Bioremediation



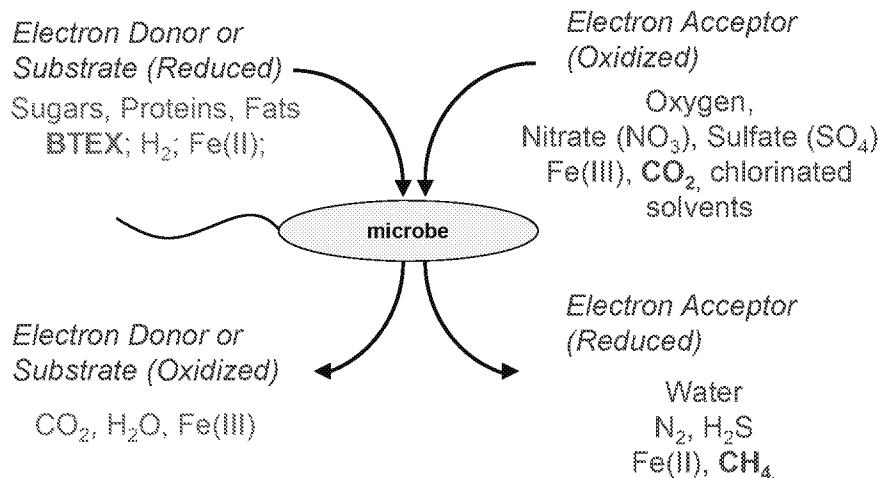
- **Biostimulation:** addition of amendments to increase biodegradation e.g., electron donors, electron acceptors, nutrients, etc.
- **Bioaugmentation:** addition of beneficial microorganisms to improve biodegradation



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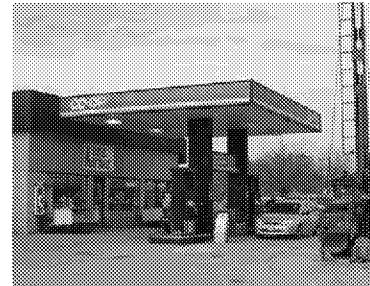
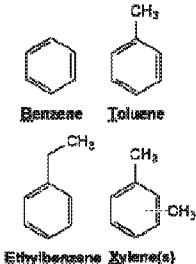
Overview of Microbial Metabolism



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BTEX Bioremediation

- Aerobic bioremediation approaches rely on delivery of oxygen.
- Intrinsic microbial populations often capable of performing aerobic biodegradation.
- When contamination is deep or under naturally induced reducing conditions aerobic bioremediation can be difficult to establish and maintain.



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BTEX Bioremediation continued...

- Biodegradation of BTEX occurs under anaerobic conditions
 - Methanogenic
 - Nitrate reducing
 - Sulfate reducing
- Microbial populations – may be present at low concentration but growth is slow
- Benzene is biggest challenge due to its unsubstituted ring structure

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Anaerobic Benzene Degradation

- Advantages of sulfate/anaerobic processes for benzene remediation
 - Sulfate highly soluble
 - Sulfate naturally present in many aquifers
 - Sulfate easier subsurface application than O₂ (e.g., Tersus Nutrisulfate®-LT)
 - Anaerobic bio less inclined towards biofouling
- Bioaugmentation culture likely required at many sites as the organisms that anaerobically degrade benzene are less ubiquitous and slow growing

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Anaerobic Benzene Culture – DGG-1



Currently Scaling
up to
Field Scale
volumes



*Anaerobic benzene seed culture
(above) benzene fermenter is
ORM2 (right)*

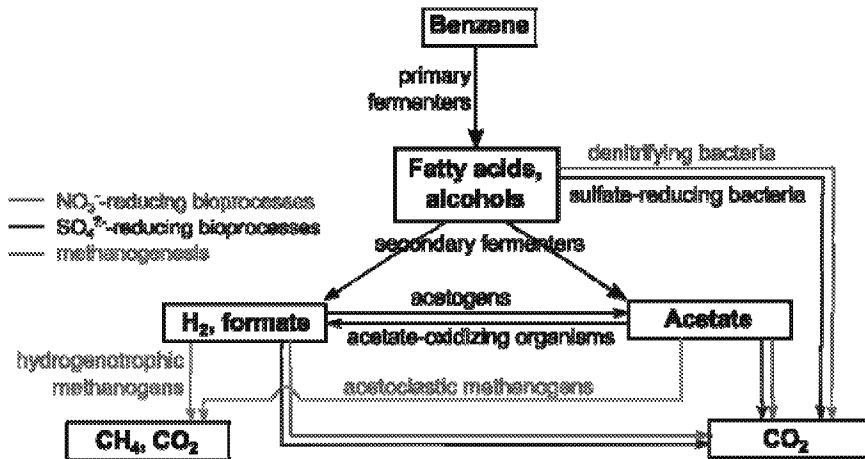
Photos Courtesy of University of Toronto

Edwards and Grbic-Galic, 1992



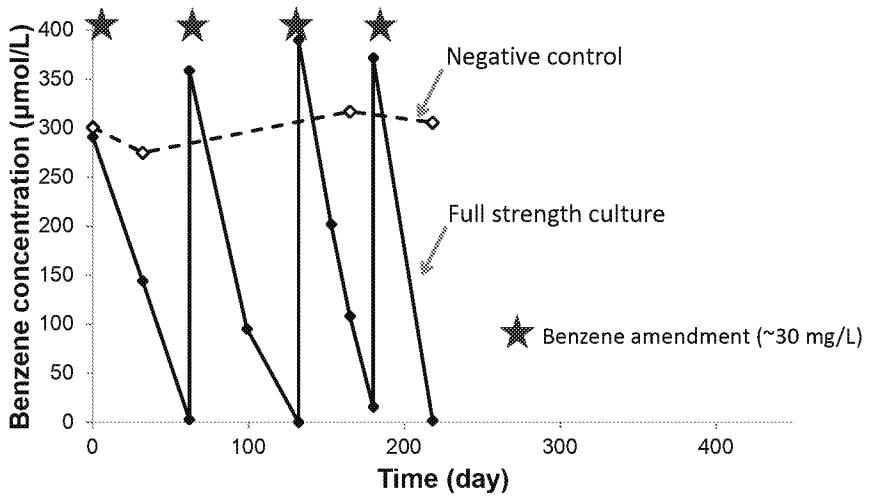
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■■■ Anaerobic Benzene Degradation



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■■■ DGG-1 Anaerobic Benzene Bioaugmentation Culture



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Characterization of Benzene Culture

Identification of key microbes in degradation pathways

- Allows identification by qPCR analysis
 - Anaerobic Benzene – ORM-2
 - Sulfate degrading bacteria - SRB

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Gene-Trac® Testing

- Are the required microorganisms indigenous to the site?
- Is bioaugmentation required?
- Impact of site amendments?
- Growth and spread of organisms in enhanced bioremediation?

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Gene-Trac® Tests Offered by SiREM

Gene-Trac Test	Target	Description
Drc	Dehalococcoides	Dechlorination of chlorinated ethenes, 1,2-DCA, PCP, TCE, DCE
TGA (tcrA/tcrC/tcrA)	VC reductases/ TCE reductase	Drc functional genes; convert TCE to DCE and cDCE & VC to ethene
Drb	Dehalococcoides	Chlorinated methane & ethane degradation
cfa	cfa and cfaV reductive dehalogenases	Dna functional genes; dechlorinase C1, 1,1,1-TCA & 1,1-DCA
Dsg	Dehalogenimonas	Dechlorinates chlorinated propenes, TCE & 1,2-DCA
Dcb	Desulfitobacterium	Dechlorinates
Pop	Geobacter	POPs, TCE-DCA, PCE/TCE, 1,2-DCA
Gcb	Geobacter	Dechlorinates PCE & TCE / long-chain hexane reduction
Polaromonas	Polaromonas substrate lyase	Aerobic CDE/VC degraders (IS-666)
cba	cbaG-cbaE	Aerobic VC degradation
1,4-Dioxane	cduCB - dioxane monooxygenase & ALDH alkylaldehyde dehydrogenase	Aerobic 1,4-dioxane degradation
DRM-2	Dehaloparaffinellum DRM-2	Respiring hexane degradation
Anammox	Planctomycetes	Ammonium and nitrate removal
SBR	Facultative reducing bacteria	Anaerobic hydrocarbon oxidation/ biodegassing reduction
Arch	Archaea	Methanogenic microorganisms
Biosurf	All Bacteria	For determining total bacterial biomass
NGS	Bacteria and Archaea	Next generation sequencing for overall microbial community composition

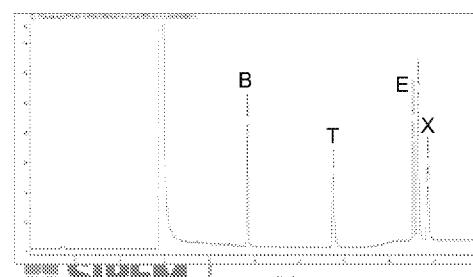
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Anaerobic Biotreatability Studies



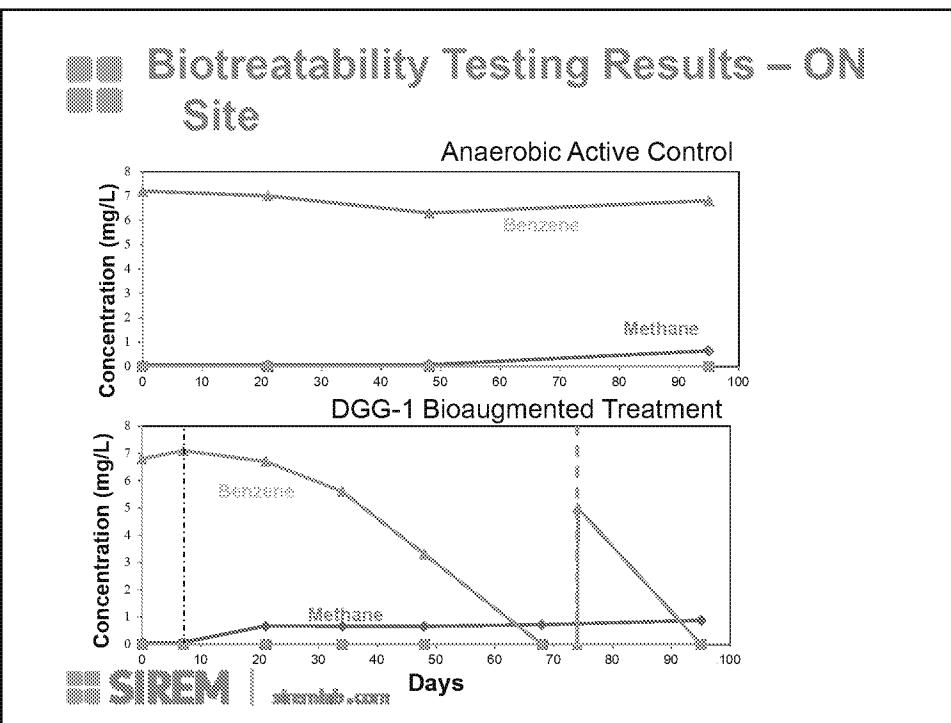
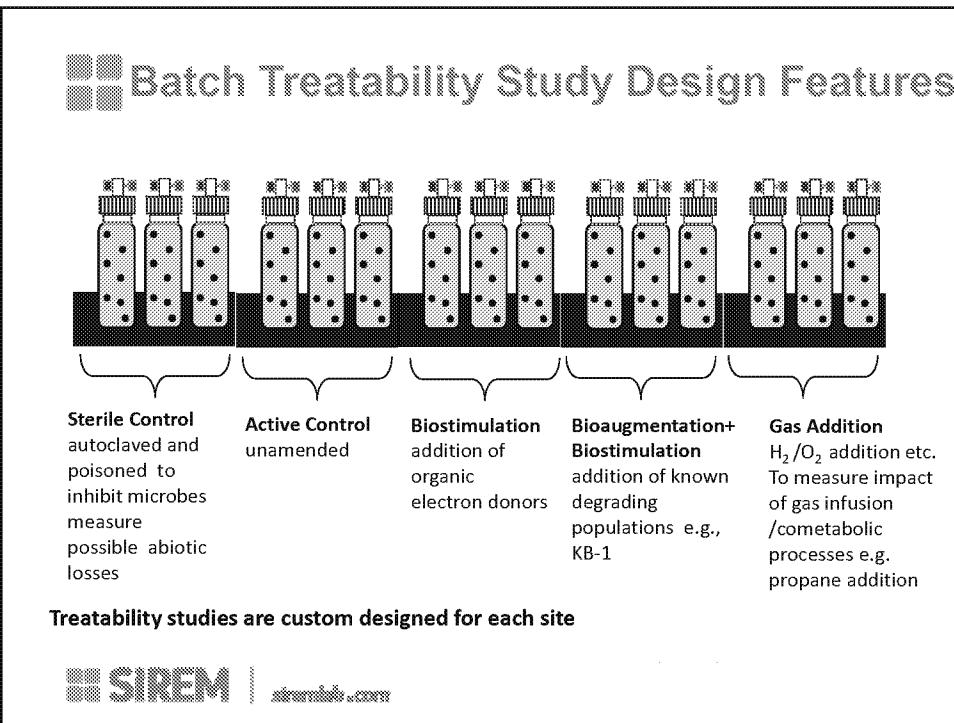
Anaerobic conditions maintained during set up incubation and sampling in glove bags filled with N₂ /CO₂ / H₂ gas mixture

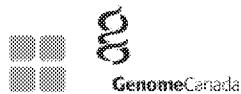


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Degradation of BTEX monitored by GC under various conditions





Anaerobic Benzene Research

- SiREM collaborator on 3 year \$750K grant with University of Toronto (Elizabeth Edwards) and Federated Co-operatives Limited

Project Goals:

- Bioaugmentation culture scale-up
- Treatability Testing
- Develop molecular genetic tests to track key organisms
- Data for regulatory approvals (safety/performance)
- Field pilot testing (Co-op site)

Do you have a benzene site? Please let us know!



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Acknowledgements

- Jennifer Webb, Peter Dollar
- Professor Elizabeth Edwards, Fei Luo, Nancy Bawa
- Federated Co-operatives

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Funding:

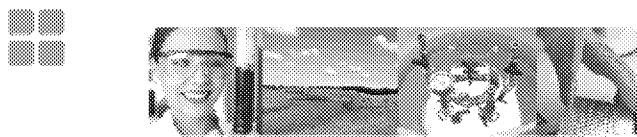


Ontario Genomics



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Thank you for attending!

Further Information

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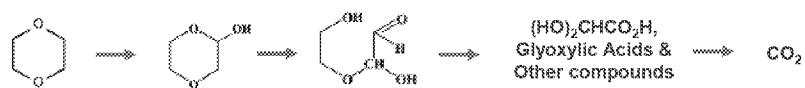
Webinar

Presenter: Dr. Shaily Mahendra
UCLA

Topic: 1,4-Dioxane

Date: 23 February 2016

Time: 12:00 – 1:00 pm EST



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